On-line Supplemental Materials

Figure S1. Employment of long frame times makes the square-well trapping potentials (in fact, all shapes of potentials in the long frame-time limit) look harmonic. (A) Trapping potential evaluated from Monte Carlo trajectories of diffusing particles totally trapped within a 720-nm length square compartment, but observed at frame times of 40 ms (solid line, European video rate) or 25 μs (dashed line). (B) Dependence of apparent diffusion coefficient on the apparent compartment size. The line is at a slope of 2. Neither of these tests performed at standard video rate are appropriate to determine the underlying potential when the diffusion of the trapped molecule is fast.

Recently, Daumas et al. (2003) reported that the results of tracking the μ-opioid receptor, a G-protein coupled receptor, in NRK cells at standard European video rate implied trapping in a quadratic potential. The width of the trapping potential was found to be about 150 nm and the microscopic diffusion coefficient was about 0.1 $\mu m^2/s$. Note that, in Daumas et al. (2003), the authors fit the MSD-t data to an equation that approaches $2L^2 + 4D_M t$ for large time, t, where L is the compartment size and D_M is the macroscopic (long-time) diffusion coefficient. Kusumi et al. (1993) showed that in the long time regime, this should approach $L^2/6 + D_M t$. Thus, the compartment size

determined by Daumas et al. (2003) should be multiplied by a factor of 3.5 ($(12)^{1/2}$) giving a corrected average potential width of 525 nm. Even after this correction, this compartment size is considerably smaller than the compartment size determined for the same cells (NRK cells) by Sako and Kusumi (1994, 1995) and Fujiwara et al. (2002), which was about 720 nm (the NRK cells have nested double compartments of ≈ 230 nm and ≈ 720 nm, but since 525 nm is closer to 720 nm, we assume that they detected these greater compartments).

Following the methods described in the text, the effective potential for a particle trapped in a square compartment of size 720 nm, with an underlying diffusion coefficient of 10 $\mu m^2/s$, is shown in Fig. S1A, observed at 25 frames per second (PAL standard frame rate). The potential looks harmonic although the true underlying potential is in fact a square potential (dashed line; also see Fig. 4 and related text). In addition, the width of this potential is comparable to that found in Daumas et al. (Daumas et al., 2003). Thus, at these long frame times, one cannot say whether the true potential is observed.

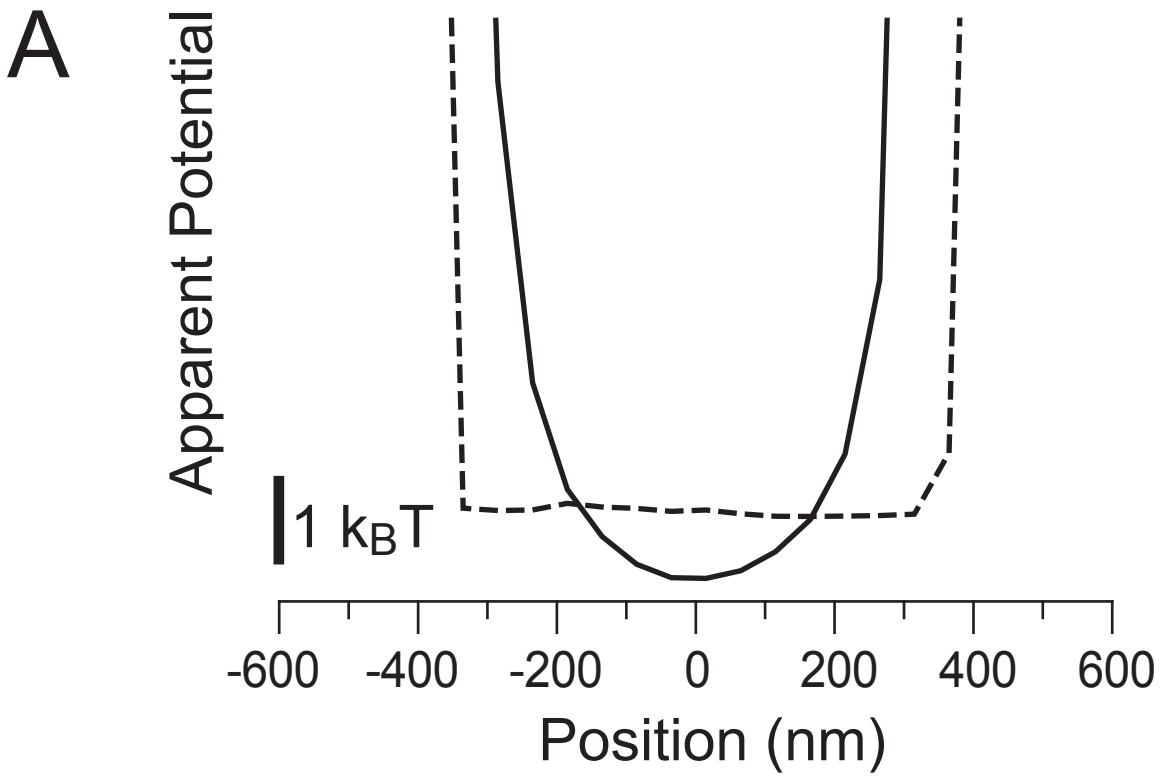
Further, Daumas et al. (2003) predict that the diffusion coefficient should vary as the confinement area, L^2, if the compartment size can vary while the number of proteins trapped within the compartment remains constant. Fig. S1B shows the results

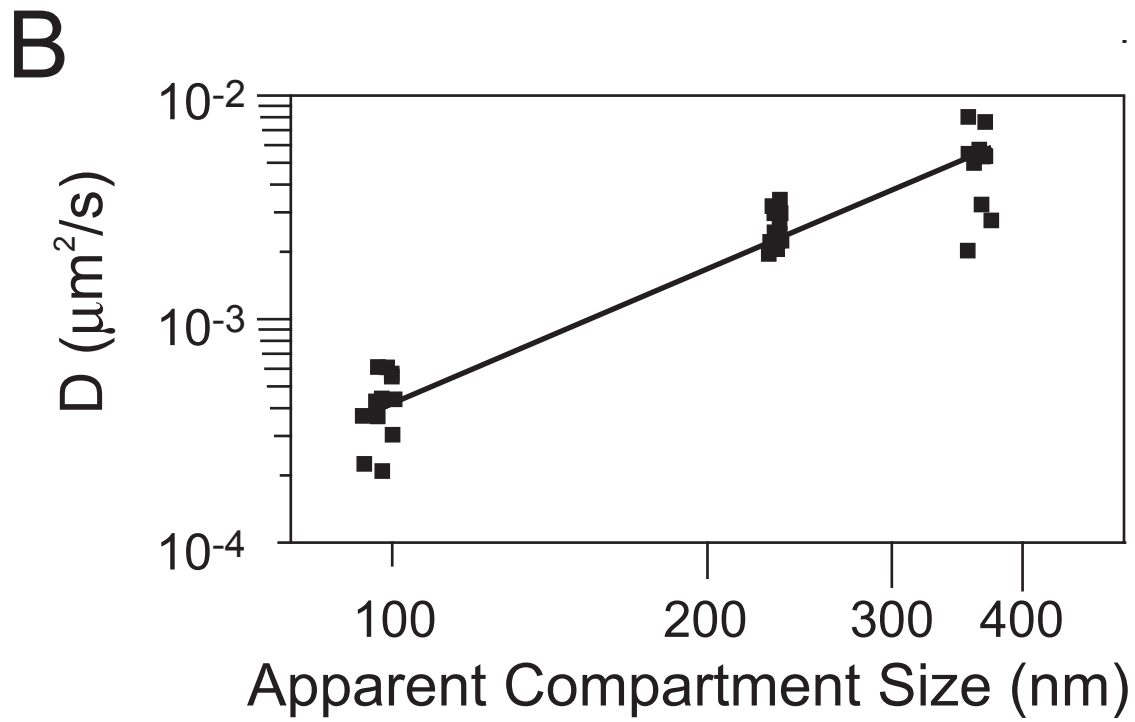
of a set of Monte Carlo simulations to simulate the effect of having a variation in the compartment sizes. The apparent microscopic diffusion coefficient, D_{2-4}, is plotted against the apparent compartment size, L, for compartments that vary in size (360, 540 and 720 nm) when the diffusion is imaged at frame exposure times of 40 ms. Here we also find a quadratic dependence of D_{2-4} on the compartment size L. This is due to the fact that both the apparent diffusion coefficient and the apparent compartment size are directly affected by the averaging that occurs during a single frame time. Thus this correlation cannot be a test of the underlying potential. By employing sufficiently short frame times, this correspondence is broken and the true diffusion coefficient is independent of compartment size.

References

Daumas, F., Destainville, N., Millot, C., Lopez, A., Dean, D., and Salome, L. 2003. Confined diffusion without fences of a G-protein-coupled receptor as revealed by single particle tracking. *Biophys. J.* 84:356-366.

Kusumi, A., Sako, Y., and Yamamoto, M. 1993. Confined lateral diffusion of membrane receptors as studied by single particle tracking (nanovid microscopy). Effects of calcium-induced differentiation in cultured epithelial cells. *Biophys. J.* 65:2021-40.





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